Investigation of gastrointestinal biomarkers in dogs with diarrhea

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Abstract

In this study, it was aimed to evaluate the zonulin and lactate levels, which are among the gastrointestinal biomarkers, in dogs with diarrhea due to various reasons. Thirty dogs with diarrhea and 15 healthy dogs, which were not classified as etiological, were included in the study. Blood samples were taken from Vena cephalica antebrachii of each dog in accordance with the technique. Serum zonulin levels were measured from the blood samples collected in tubes without anticoagulant, using the commercial test kit based on the ELISA principle. Plasma lactate levels were measured using a handheld analyzer from blood samples taken into heparinized tubes. While serum zonulin concentrations were found to be 9.80 ± 6.7 ng/mL in dogs with diarrhea, serum zonulin concentrations were found to be 1.94 ± 1.4 ng/mL in healthy dogs. It was determined that the plasma lactate levels of the dogs with diarrhea were 9.02 ± 4.7 mmol/L, whereas the plasma lactate concentrations of the healthy dogs were 1.21 ± 1.4 mmol/L. In the statistical evaluation, both zonulin and lactate concentrations were found to be highly significant ($p< 0.05$) for dogs with diarrhea. As a result, it was concluded that the gastrointestinal biomarkers zonulin and lactate levels increased in blood in dogs with diarrhea and that zonulin and lactate could be taken into account in detecting the damage that may occur in the intestines due to diarrhea, and these markers could be used in the follow-up of the prognosis and treatment of diarrhea.

Keywords: *Diarrhea, dog, lactate, zonulin*

INTRODUCTION

Gut health not only contributes to the prevention of various diseases but has also become a significant focus among nutritionists, veterinarians, and scientists (Kogut and Arsenault, 2016). Generally, gut health is discussed under six main areas: diet, digestion and absorption, normal and balanced microbiota, immune status, intestinal mucosa, and neuroendocrine-gut motor functions. Understanding these functions plays a crucial role in animal health, welfare, and performance (Celi et al., 2017). Diarrhea, the primary indication of impaired gut health in dogs, notably affects young dogs and is one of the leading causes of mortality in this age group (Mila et al., 2017; Münnich and Küchenmeister, 2014). Diarrhea in dogs is categorized as infectious or non-infectious based on etiology, and as acute (lasting less than 14 days) or chronic based on duration, making this classification highly relevant both clinically and for research (Nind, 2011; Schulz et al., 2008; Volkmann et al., 2017; Willard, 2013). The pathogenesis of diarrhea involves absorption disorders associated with damage to the villi and microvilli structures in the small intestine, while in the large intestine, it is linked to an increase in fecal water content due to inadequate water and electrolyte absorption by colonocytes. Moreover, the fermentation of indigestible nutrients by lactic acid bacteria can increase the osmolarity of intestinal contents, potentially exacerbating diarrhea (Burrows et al., 1995; Heyman, 2000; Volkmann et al., 2017). The primary pathology underlying intestinal diseases is leaky gut syndrome, caused by the disruption of the mucosal barrier and the resulting increase in intestinal permeability. Tight junction proteins between intestinal epithelial cells play a critical role in regulating the mucosal barrier, with the zonulin protein controlling the permeability of these connections (Sturgeon and Fasano, 2016). An increase in zonulin levels can lead to heightened intestinal permeability, contributing to the development of inflammatory, autoimmune, and neoplastic diseases. Disruption in the zonulin signaling pathway can alter immune responses and weaken mucosal tolerance, thereby playing a role in the pathogenesis of chronic inflammatory diseases (Fasano, 2012a). Both chronic and acute inflammatory conditions are marked by changes in intestinal permeability and dysbiosis, closely associated with compromised gut integrity. This disruption can allow microorganisms to enter the circulation through the gut, potentially triggering irregular immune responses that result in sepsis, septic shock, and irreversible organ damage (Koh et al., 2006; Vaishnavi, 2013). Hypovolemia and hypoperfusion, frequently observed in sepsis and septic shock, lead to impaired tissue oxygenation, triggering anaerobic metabolism and causing excessive lactate production (Shahrin et al., 2024). As a byproduct of bacterial fermentation, D-lactate levels increase in association with ischemic gut injury and enhanced gut permeability. This increase in D-lactate, released into the portal and systemic circulation, has been linked to D-lactic acidosis in conditions such as short bowel syndrome and exocrine pancreatic insufficiency. The aim of this study is to evaluate lactate and zonulin levels in dogs with acute diarrhea and to examine the relationship of these parameters with intestinal permeability and metabolic responses.

MATERIALS AND METHODS

Animal material

The animal material for this study consisted of 30 dogs of different ages and sexes presenting with diarrhea of unclassified etiology at the Small Animal Clinic of the Department of Internal Medicine, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, and 15 he-

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althy dogs determined to be clinically, hematologically, and biochemically normal. An additional criterion for including the diarrheic animals in the study was that they had not received antibiotics for at least one month, to ensure that zonulin levels would accurately reflect disrupted intestinal permeability.

Sampling procedure

In this study, blood samples were collected from each dog's *vena cephalica* antebrachii into heparinized tubes and serum tubes in accordance with proper techniques. Blood samples in serum tubes were centrifuged for 15 minutes at 3000 rpm to obtain serum samples. The serum samples were stored at -20°C until analysis.

Laboratory analysis

Zonulin and lactate analysis

Serum zonulin levels were determined using a Canine Zonulin ELISA Kit (Cat No. MBS2605074). The results from the test kit were evaluated using an ELX800 microplate reader. Plasma lactate levels were measured using a handheld analyzer (Lactate Pro 2, Arkray, Netherlands).

Statistical analyses

Descriptive statistics were conducted on the numerical data obtained in the study and presented in Table 1. The Shapiro-Wilk was used to check data distribution, revealing that distributions were not normal. Although all data underwent logarithmic (Logn) transformation, the distributions remained non-normal. Therefore, differences between groups were analyzed using the non-parametric Mann-Whitney U test. Data on lactate and zonulin levels were illustrated with box plots. Age and gender information for the healthy and diarrheic dogs was determined using cross-tabulation. All analyses were performed using SPSS® software (version 20.0, IBM, USA), and statistical significance was set at $p < 0.05$

RESULTS

Among the diarrheic dogs included in the study, 14 (46.6%) were male, and 16 (53.4%) were female. Based on age distribution, 18 dogs (60%) were younger than one year, and 12 dogs (40%) were between one and five years old. In the healthy group, gender distribution was balanced, with 7 males and 8 females. The age distribution of healthy dogs showed that 5 were under one year old, 9 were between one and five years, and 1 was older than five years. No statistically significant difference was found in age or gender distribution between healthy and diarrheic dogs. Serum zonulin levels in diarrheic dogs were significantly higher than in healthy dogs ($p < 0.05$). These differences were also depicted in Table 1. When examining lactate levels between diarrheic dogs and healthy dogs, a significant statistical difference was detected ($p < 0.05$). Plasma lactate levels were measured at 9.02 ± 4.7 mmol/L in diarrheic dogs and 1.21 ± 1.4 mmol/L in healthy dogs. Serum zonulin concentrations were determined to be 9.80 ± 6.7 ng/mL in diarrheic dogs and 1.94 ± 1.4 ng/mL in healthy dogs.

DISCUSSION

The identification of diet, digestion, absorption, microbiota balance, immune status, intestinal mucosa, and neuroendocrine-gut motor functions is crucial for monitoring animal health and welfare and assessing the impact of nutritional interventions on animal performance (Celi et al., 2017). Diarrhea is a clinical symptom characterized by an increase in both the fluid content and volume of feces, which can arise from infectious or non-infectious causes. In this study, no etiological classification was made for diarrheic dogs. However, it was noted that diarrhea, particularly in young dogs with immature immune systems, can lead to serious consequences (Mila et al., 2017; Münnich et al., 2014). The finding that 18 of the 30 diarrheic dogs in our study were under one year of age supports this observation. In recent years, the importance of gut health has grown among nutritionists, veterinarians, and scientists (Kogut and Arsenault, 2016). However, there remains a substantial gap in the identification of biomarkers related to gastrointestinal barrier function, permeability, and the gut endocrine system (Celi et al., 2017). Tight junctions between intestinal epithelial cells play a critical role in regulating the mucosal barrier, with zonulin acting as a key protein that reversibly regulates the permeability of these connections. Numerous clinical studies have examined zonulin as a biomarker of intestinal permeability (Fasano, 2012a; Sturgeon and Fasano, 2016). As another biomarker, lactate is present in the D-enantiomer form, typically undetectable in mammalian serum under normal physiological conditions. However, it may increase in serum due to bacterial fermentation in the gastrointestinal tract or as a result of metabolic disorders (Christopher et al., 1990). Increased gut permeability and bacterial overgrowth can lead to elevated circulating D-lactate levels, which, like zonulin, is thus regarded as a significant biomarker of gastrointestinal dysfunction (Peoc'h et al., 2018). In our study, we found statistically significant differences in zonulin and lactate levels between diarrheic dogs and the healthy control group ($p < 0.05$). Specifically, zonulin levels in diarrheic dogs were 9.80 ± 6.7 ng/mL, and serum lactate levels were 9.02 ± 4.7 mmol/L, both significantly higher than in the control group. The gut serves as a critical barrier against harmful substances in the body. Disruption of this barrier and increased permeability can allow pathogens to enter the circulatory system and lead to gastrointestinal symptoms like diarrhea (Fasano, 2012a). Zonulin plays a crucial role in the pathogenesis

Table 1. *Statistical comparison of serum lactate and serum zonulin level*

Group	Lactate (mmol/L)	Zonulin (ng/mL)
Diarrheal	9.02 ± 4.7	9.80 ± 6.7
Healthy	1.21 ± 1.4	1.94 ± 1.4
p-value	0.05	0.05

of many chronic inflammatory bowel diseases (Sturgeon and Fasano, 2016). In our study, high zonulin levels detected in diarrhea cases with various etiologies indicate a direct relationship between this biomolecule and gut health. Moreover, studies in the literature indicate that elevated zonulin levels are associated with systemic diseases such as autoimmune diseases, type 1 diabetes mellitus, and celiac disease (Fasano and Shea-Donohue, 2005; Fasano, 2012b). It has been shown that intestinal permeability begins to increase in individuals prone to type 1 diabetes before the onset of disease symptoms (De Magistris et al., 1996). Arrieta et al. (2009) demonstrated in a mouse study that treatment with the zonulin inhibitor AT-1001 reduced gut permeability, thereby preventing colitis. Zonulin has also been linked to asthma and brain tumors (Díaz-Coránguez et al., 2013; Fasano, 2011). Similar to findings in the aforementioned literature, we observed significant increases in zonulin levels in dogs with acute diarrhea in our study. These findings suggest that zonulin could serve as a biomarker for assessing intestinal permeability in dogs. Lactate, a byproduct of anaerobic glycolysis, is particularly elevated in serum in cases of shock, anemia, respiratory disorders, and gastrointestinal abnormalities (Fall and Szerlip, 2005). Elevated lactate levels are generally considered an indicator of hypoxia and tissue hypoperfusion (Kruse and Carlson, 1987). Besides hypoperfusion and hypoxia, lactate levels may also increase due to various drugs, toxins, mitochondrial defects, and conditions like sepsis (Kruse and Carlson, 1987; Luft, 2001). Chronically elevated lactate levels have been linked to an increased risk of mortality in clinical settings (Hayes et al., 2010, 2011). In this study, the significantly elevated total serum lactate levels in diarrheic dogs serve as an important indicator of increased intestinal permeability and anaerobic metabolism. Studies have shown that in cases with high plasma lactate levels, the mortality rate remains elevated despite treatment (Cortellini et al., 2015; Stevenson et al., 2007). While our study did not monitor mortality in dogs with high lactate levels, these findings underscore the need for future research examining the relationship between lactate levels and mortality.

CONCLUSION

Both biomarkers appear clinically valuable, especially for monitoring prognosis and treatment response in diarrhea cases. Furthermore, we conclude that treatment protocols should avoid medications that could negatively impact gut permeability and mitochondrial function. The routine use of zonulin and lactate levels in veterinary clinics may aid in identifying the underlying pathophysiological mechanisms of diarrhea.

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Conflict of interest

The authors declare no conflict of interest regarding this article.

Ethical stamement

This study was reviewed and approved by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (ADU-HADYEK) in accordance with ethical principles. The ethical approval was granted on 09/07/2020, with the approval number 64583101/2020/036.

Author contributions

Idea/Concept: SP; Design: SP, TŞ; Control/Supervision: SP, TŞ, TÖ; Data Collection and/or Processing: SP, TŞ; Analysis and/or Interpretation: SP, TŞ; Literature Review: SP, TŞ, TÖ; Writing the Article: SP, TŞ, TÖ; Critical Review: SP, TŞ, TÖ; References and Fundings: SP, TŞ; Materials: SP, TŞ.

Availability of data and materials

The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request. All software and materials used in this study are commercially available or described in detail in the Materials and Methods section. Data supporting the findings of this study are included in the main text and supplementary materials.

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